REMARKS

The Official Action dated February 5, 2003 has been carefully considered. Accordingly, the changes presented herewith, taken with the following remarks, are believed sufficient to place the present application in condition for allowance. Reconsideration is respectfully requested.

By the present Amendment, claims 4 and 5 are amended to include limitations from the specification, for example in the last sentence at page 6 and the first sentence at page 7. Claim 6 is cancelled and the dependency of claims 7-11 is changed from claim 6 to claim 22. It is believed that these changes do not involve any introduction of new matter, whereby entry is believed to be in order and is respectfully requested.

Claims 4, 5, 7-11 and 18-23 are pending in this application. The Examiner previously indicated that claims 22 and 23 were allowable. As the dependency of claims 7-11 has been changed to claim 22 and claims 18-21 depend from claim 8, it is believed that claims 7-11 and 18-21, now directly or indirectly dependent on claim 22, are also allowable. Reconsideration is respectfully requested.

In the Official Action, claim 4 was rejected under 35 U.S.C. §102(b) as being anticipated by Chemical Abstract 87:63008. The Examiner asserted that the chemical abstract teaches use of the claimed prostaglandin in a pharmaceutical formulation as a bronchodilator.

However, Applicants submit that the composition of claim 4 is not anticipated by and is patentably distinguishable from the teachings of the cited chemical abstract. Accordingly, this rejection is traversed and reconsideration is respectfully requested.

More particularly, the cited chemical abstract discloses the synthesis and brochodilator activity of DL-16,16-trimethylene prostaglandins. On the other hand, claim 4 is directed to a composition for the treatment of glaucoma and ocular hypertension, which

composition comprises a therapeutically active and physiologically acceptable amount of 15(R,S)-16,16-trimethylene PGE_2, or an alkyl ester thereof, or a pharmaceutically acceptable salt thereof, and an ophthalmologically-compatible vehicle. Additionally, the composition is adapted for topical application to the eye. Applicants find no teaching or suggestion in the cited chemical abstract relating to a composition containing a prostaglandin in combination with an ophthalmologically-compatible vehicle, or relating to a composition adapted for topical application to the eye.

Anticipation under 35 U.S.C. §102 requires that each and every element as set forth in the claims is found, either expressly or inherently described, in a single prior art reference, *In re Robertson*, 49 U.S.P.Q.2d 1949, 1950 (Fed Cir. 1999). In view of the failure of the cited chemical abstract to teach the combination of a prostaglandin as presently claimed and an ophthalmologically-compatible vehicle, and the failure of the cited chemical abstract to teach a composition adapted for topical application to the eye, the cited chemical abstract fails to disclose each and every element set forth in claim 4. Thus, the chemical abstract does not anticipate claim 4 under 35 U.S.C. §102. It is therefore submitted that the rejection has been overcome, and reconsideration is respectfully requested.

Claims 5-11 and 18-21 were rejected under 35 U.S.C. §102(b) as being anticipated by the Stjernschantz et al U.S. Patent No. 5,296,504. The Examiner asserted that Stjernschantz et al teach the use of the claimed prostaglandins in a pharmaceutical formulation for the treatment of glaucoma.

As noted above, claim 6 has been cancelled and the dependency of claims 7-11 and 18-21 has been changed, directly or indirectly, to allowed claim 22. It is therefore believed that the present rejection relates only to present claim 5. However, Applicants submit that the composition defined by claim 5 is not anticipated by and is patentably distinguishable from

the teachings of Stjernschantz et al. Accordingly, this rejection is traversed and reconsideration is respectfully requested.

More particularly, claim 5 is directed to a composition for the treatment of glaucoma and ocular hypertension. The composition comprises a therapeutically effective and physiologically acceptable amount of a prostaglandin analog which is a selective agonist for EP_1 prostanoid receptors, or a pharmaceutically acceptable salt or ester thereof, and an ophthalmologically-compatible vehicle. The prostaglandin analog is 13,14-dihydro-17-(3-fluorophenyl)-18,19,20-trinor-PGE_2 or an alkyl ester thereof. The composition is adapted for topical application to the eye.

Stjernschantz et al disclose prostaglandin derivatives for the treatment of glaucoma or ocular hypertension. Derivatives of PGA, PGB, PGD, PGE and PGF, in which the omega chain contains a ring structure, are disclosed. Numerous compounds are covered by the generic formula which Stjernschantz et al disclose. However, Applicants find no specific teaching by Stjernschantz et al relating to the prostaglandin analogue included in the composition of claim 5, namely 13,14-dihydro-17-(3-fluorophenyl)-18,19,20-trinor-PGE_2 or an alkyl ester thereof. Additionally, Applicants find no teaching by Stjernschantz et al relating to prostaglandin analogues which are selective agonists for EP_1 prostanoid receptors. In fact, Applicants advise that compounds similar to that of claim 5, for example 13,14-dihydro-17-phenyl-18,19,20-trinor-PGF_2 alpha, the free acid of latanoprost, the active ingredient of Xalatan, is a prostaglan FP receptor agonist (see, for example, the attached Stjernschantz et al, *Drugs of the Future*, 17(8):691-704 (1992), particularly page 699), as is 16-phenoxy(3-trifluoromethyl)-17,18,19,20-tetranor-PGF_2 alpha, the free acid of travapost, the active ingredient of Travatan.

Thus, not only is the prostaglin analogue in the composition of claim 5 not specifically disclosed by Stjernschantz et al, it exhibits a selective agonist property different

from similar compounds specifically disclosed by Stjernschantz et al. In view of the failure of Stjernschantz et al to specifically teach the prostaglandin analog included in the composition of claim 5, and to recognize the selective agonist for EP_1 prostanoid receptor activity exhibited thereby, Stjernschantz et al do not disclose each and every element of claim 5. Thus, Stjernschantz et al do not anticipate claim 5 under 35 U.S.C. §102, *In re Robinson, supra*. It is therefore submitted that the rejection has been overcome. Reconsideration is respectfully requested.

It is believed that the above represents a complete response to the rejections under 35 U.S.C. §102 and places the present application in condition for allowance. Reconsideration and an early allowance are requested.

Respectfully submitted,

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Drugs of the Future 1992, 17(8): 691-704

Correlates in Pharmacostructures

Ph nyl substituted prostaglandin analogs for glaucoma treatment

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Phenyl substituted prostaglandin analogs for glaucoma tr atment

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Introduction

In the eye prostaglandins have generally been associated with inflammation. This misconception goes back to the late 1960s and 1970s and was, to a large extent, due to studies designed to prove the inflammatory role of prostaglandins in the eye. One problem with these studies was that large quantities of prostaglandins were administered to the eye, and usually by direct injection. Another problem was that in many of the studies, rabbits were used as experimental animals. The rabbit eye is prone to reacting to a variety of stimuli with increased blood flow and disruption of the blood-aqueous barrier in the anterior uvea. This protective mechansim of the rabbit eye (1) is in sharp contrast to primate and human eyes, which generally are much less sensitive to trauma. It is conceivable that endogenous prostaglandins may play an important role in this protective m hanism of the rabbit eye.

The first study to demonstrate a clear-cut reduction in intraocular pressure (IOP) after topical administration of prostaglandins was that of Camras *et al.* (2). In this study a biphasic response in IOP could be obtained with small doses of prostaglandins, *e.g.*, prostaglandin F_{2alpha} (PGF_{2alpha}) 1 (Scheme 1); first an increase and then a sustained decrease. A topical dose of 5 mcg induced only a decrease in IOP (2). Unfortunately, however, this study was performed in rabbits, a species exhibiting marked tachyphylaxis to prostaglandins, and is therefore not representative for the human and primate eye in which prostaglandins lower IOP by another mechanism of action. In subsequent studies it has been demonstrated that prostaglandins indeed reduce IOP in primates and cats as well as in dogs (3-10).

The most relevant animal model with respect to the human eye is the monkey eye and mechanism studies performed with prostaglandins in monkeys will therefore be described. Several independent studies clearly indicate that the main mechanism of action to reduce IOP, at least of PGF_{2alpha} and its isopropyl ester, is increased uveoscleral outflow of aqueous humor (11-14). Aqueous humor is produced in the ciliary processes behind the iris. It then flows through the pupil from the posterior chamber into the anterior chamber between the iris and the cornea (Fig. 1). Normally, most of the aqueous humor exits the eye through the trabecular meshwork and Schlemm's canal situated in the chamber angle. Schlemm's canal is directly connected to episcleral veins outside the eye. However, part of the aqueous humor bypasses this exit route and leaves the eye through the so-called uveoscleral outflow pathway (15). In this pathway aqueous humor percolates through the ciliary muscle from the anterior chamber to enter into the supraciliary and suprachoroidal spaces from which the fluid relatively easily can pass out from the eye through the sclera (Fig. 1). The main resistance in this pathway is constituted by the ciliary muscle.

In glaucoma the drainage of aqueous humor from the anterior chamber is obstructed in the trabecular meshwork and/or the tissue adjacent to Schlemm's canal. Thus, if part of the fluid could be shunted out from the eye through another route this would be very attractive from a pathophysiological and clinical point of view. In fact, theoretically, since the pressure gradient forcing fluid into the uveoscleral outflow pathway is very small, if all aqueous humor were to exit the eye through this route an IOP close to the episcleral venous pressure would ensue. Such a pressure level, around 10 mmHg, would be very desirable in glaucoma management.

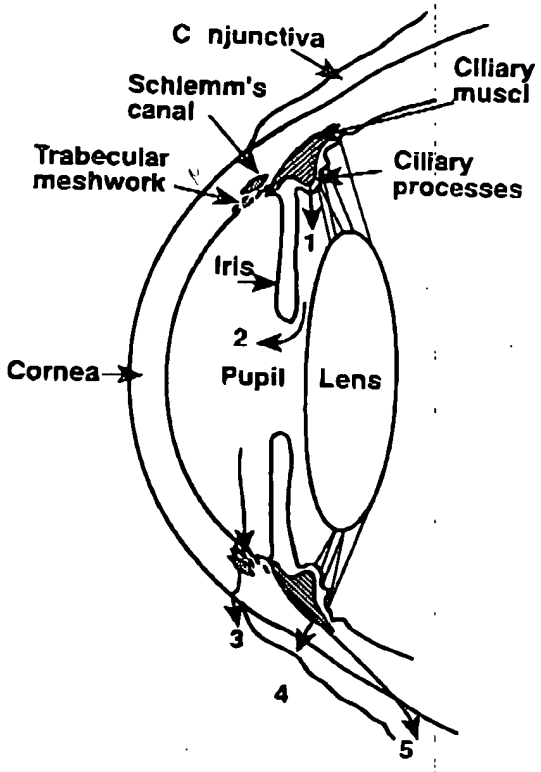


Fig. 1. Schematic picture of the anterior segment of the eye. The aqueous humor dynamics in the anterior segment determines the intraocular pressure together with the pressure in the blood vessels outside the eye. Aqueous humor is produced in the ciliary processes (1). It flows through the pupil into the anterior chamber (2), through the trabecular meshwork into Schlemm's canal and out into the blood vessels on the surface of the eye (3). Part of the aqueous humor exits through the uveoscleral pathway, traversing the ciliary muscle to enter into the supraciliary and suprachoroidal spaces, from where the fluid can leave the eye through the sclera (4 and 5, respectively).

PGF_{2alpha} and PGF_{2alpha} isopropyl ester (PGF_{2alpha}-ie) 2 (Scheme 1) have been shown effectively to reduce IOP both in normotensive healthy volunteers and in patients suffering from ocular hypertension or open angle glaucoma (16-23). However, both PGF_{2alpha} and PGF_{2alpha}-ie cause pronounced local side effects when applied topically on the eye. A diester prodrug, 15-propionate-PGF_{2alpha}-ie, was not found to significantly improve the therapeutic index of PGF_{2alpha} in the eye of human volunteers (24). These side effects comprise superficial irritation, mostly experienced as a grittyness or foreign body sensation and conjunctival hyperemia lasting for several hours (25). Because of the side effects it has not been possible to develop PGF_{2alpha} or an esterified prodrug of PGF_{2alpha} to a useful drug for glaucoma treatment in spite of the very good IOP lowering effect of this prostaglandin. It should be stressed, however, that PGF_{2alpha} and PGF_{2alpha}-ie have never been found to induce any intraocular side effects, and therefore from a clinical point of view, this class of drugs probably would be acceptable as long as the superficial ocular side effect profile is improved.

Attempts were made to reduce the local side effects of prostaglandins by a prodrug concept through esterification of different parts of the molecule. Esterification increases lipophilicity of the molecule and thus the bioavailability in the eye. The sites of esterification of PGF_{2alpha} used for the prodrug concept are illustrated in Scheme 1. These prodrugs of PGF_{2alpha} were prepared in the early 1980s. Unfortunately, the prodrugs did not significantly increase the therapeutic index of PGF_{2alpha} in the eye. However, substituting part of the omega chain with a phenyl ring (Scheme 2) has been shown to change the pharmacological profile of PGF_{2alpha} dramatically with respect to the side effects in the eye (26-29).

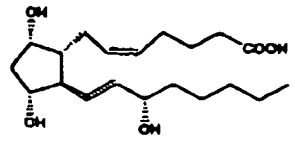
General method for synthesis of phenyl substituted PGF_{2alpha} analogues

The omega chains of the phenyl substituted PGF_{2alpha} analogues were synthesized from the appropriate phosphoranes (30-32) or phosphonates (33) as key reagents. Three general routes were utilized as outlined in Scheme 3.

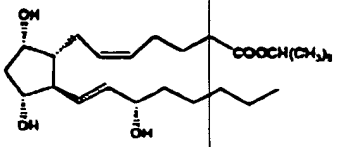
The acyl triphenylphosphorane I (Scheme 3) was prepared by addition of an aryl halide to lithiotriphenyl phosphinoacetonide (method A) or by reaction of methyl triphenylphosphonium bromide with aryl acid ester using potassium *t*. butoxide (method B). The reactive dimethyl (2-oxoalkyl) phosphonates were prepared by reaction of aryl halide and dimethyl (2- oxopropyl) phosphonate in THF using *n*-BuLi (method C). These precursors were prepared in 55-60% yield.

The phenyl substituted PGF_{2alpha} analogues were prepared from a commercially available bicyclic lactone (34, 35) corresponding to formula III as outlined in Scheme 4. The primary alcohol of lactone III was oxidized to aldehyde IV using dimethyl sulfoxide (DMSO) and dicyclohexylcarbodiimide (DCC) in the presence of anhydrous phosphoric acid in dimethoxyethylene (DME) (36-38). The crude aldehyde IV was reacted with the appropriate acyl phosphorane or acyl phosphonate I, II (Scheme 3) using a method described by Emmon-Horner (39, 40) affording alpha, beta unsaturated ketone V. The resulting enone V was treated with lithium tri *sec* butylboro hydride (lithium selectride) (41) at -120°C/-130°C, furnishing 70-75% *S* isomer VIa over *R* isomer VIb. Sodium borohydride and cerium chloride (41) were also used but with lower stereoselectivity. The isomers were separated by column chromatography on silica gel using toluene: AcOEt 2:1 as eluent. The phenyl benzoyl group was removed by using powdered potassium carbonate in methanol to give an 80% yield of the diol. The product was purified by column chromatography on silica gel using AcOEt as eluent. The diol was treated with diisobutyl aluminium hydride (DIBAL) (42) in dry THF at -78°C to afford lactol (triol) VII in 75-80% yield. The triol VII underwent Wittig reaction with 4-carboxy butyl triphenylphosphonium bromide and KotBu in THF furnishing the phenyl PGF_{2a} acid VIII. This was further reacted without isolation with isopropyl iodide (iprI) and DBU in acetone (43) to give the corresponding ester in about 50% yield. The 15-allylic alcohol of the phenyl PGF_{2alpha} ester IX was oxidized with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) (44, 45) in dioxane to give the

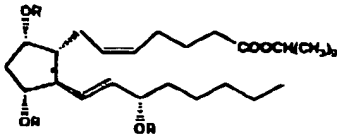
Scheme 1



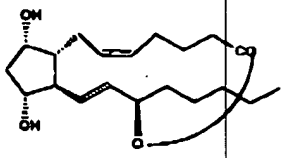
PGF2α
1



PGF2α Ic
2



R=H, COCH3
3



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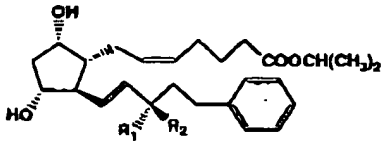
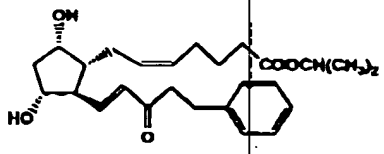
Scheme 2



5- R1=OH R2=H
6- R1=H R2=OH



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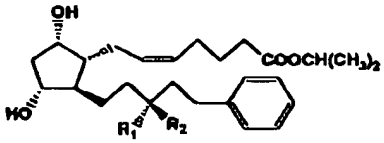
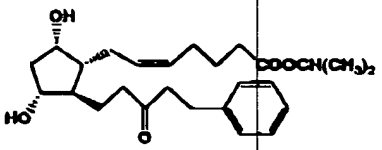


8- R1=OH R2=H
9- R1=H R2=OH



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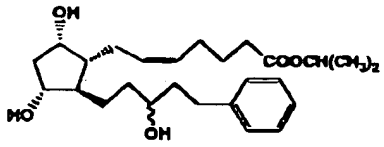
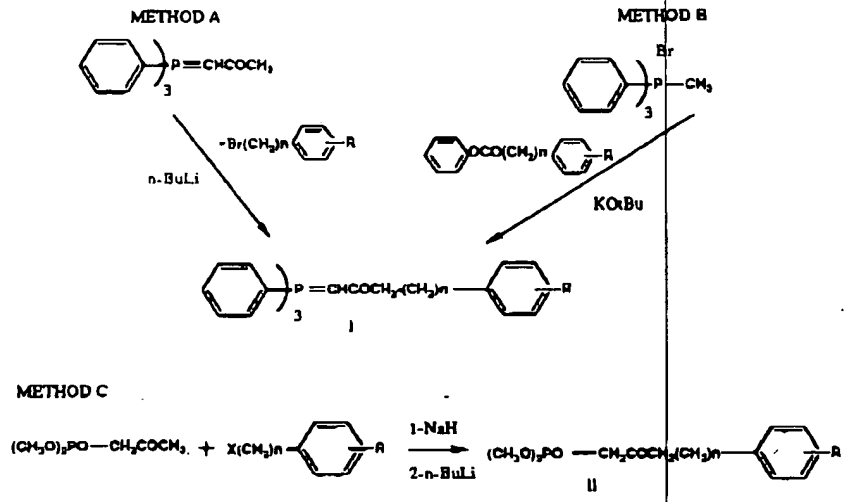


11

desired 15-keto phenyl PGF_{2alpha} analogue X in about 80% yield.

13,14-dihydro phenyl PGF_{2alpha} analogues were synthesized as outlined in Scheme 5. The *trans* allylic double bond of compound VI was reduced under hydrogen atmosphere using pd-c as a catalyst in the presence of sodium nitrite (46) affording compound XI in quantitative yield. The product XI was isolated and reacted subsequently following a procedure described above to give the desired product XII (Schemes 4 and 5). The 9,11 dihydroxyl groups of the phenyl PGF_{2alpha} analogue XII were protected with benzene boronic acid (47) to give 9,11-phenyl boronate, which was further reacted without isolation with pyridinium chlorochromate (PCC) adsorbed on alumina (48) in CH_2Cl_2 to give the 15-keto analogue XIII. This was treated with hydrogen peroxide to deprotect the 9,11-phenyl boronate,

Scheme 3



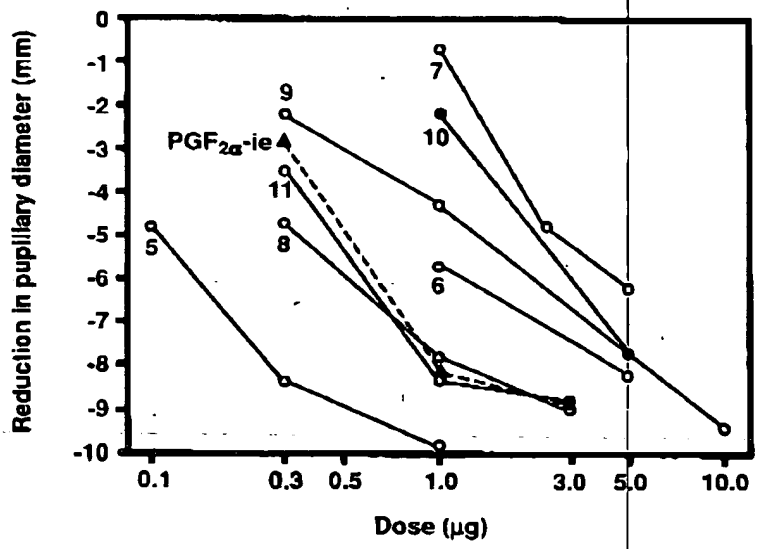
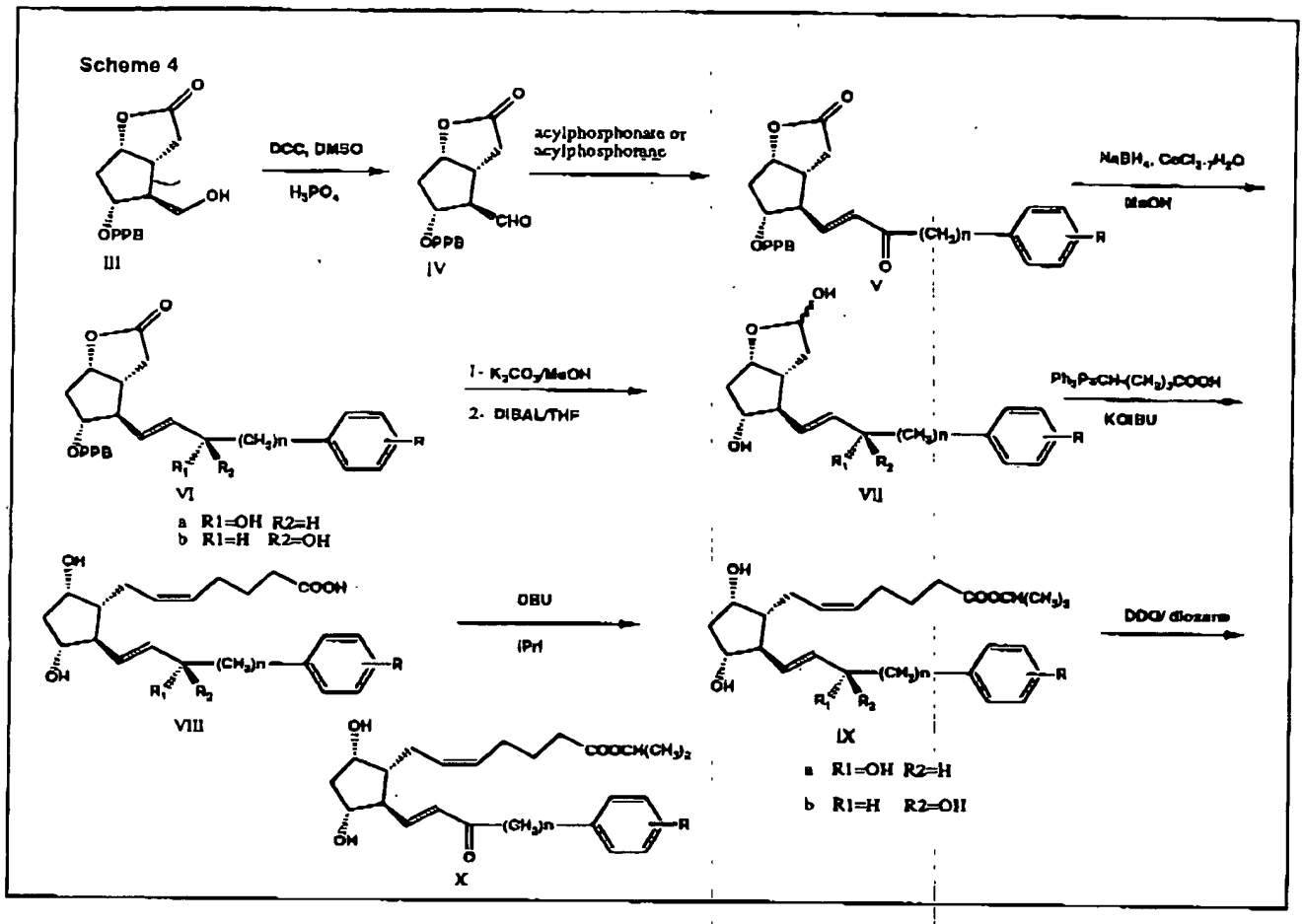


Fig. 2. Miotic effect of 17-phenyl substituted PGF_{2alpha}-ie analogues in cat eyes 3 hours after topical application (maximum effect). PGF_{2alpha}-ie included for comparison (n = 6).



giving the desired product, the 15-keto phenyl PGF_{2alpha} analogue XIV in good yield.

The analogues were identified with ^{13}C and ^{1}H NMR and the purity was determined with HPLC. All analogues were used as isopropyl esters to enhance bioavailability in the eye.

17-Phenyl substituted PGF_{2alpha} analogues

Structure-activity relationships of 17-phenyl substituted PGF_{2alpha}-ie analogues

The test compounds were administered topically on the eye in aqueous solution. All the 17-phenyl substituted PGF_{2alpha}-ie analogues (Scheme 2) exhibited marked and dose dependent miotic (pupillary constrictive) effect in the cat (Fig. 2). The horizontal pupillary diameter of the experimental eye was compared with that of the contralateral control eye treated with vehicle only. In fact, some of the phenyl substituted analogues such as compound **5** (Scheme 2) were more potent than PGF_{2alpha} (administered as the isopropyl ester),which is endogenous in the eye (Fig. 2). These results seem to be in fairly good agreement with those of previously reported studies with 17-phenyl-18,19,20-trinor-prostaglandins in other biological systems (49). In spite of the fact that PGF_{2alpha}-ie is very irritative in the cat eye, none of the phenyl substituted PGF_{2alpha}-ie analogues caused any ocular irritation as judged from the behavior of the animals as well as from the degree of lid closure after topical administration of the compunds (Table I). The marked miotic effect of these compounds in combination with the total lack of irritative effect strongly suggests that substitution of part of the omega chain with an aromatic ring structure either causes conformational alteration in the molecule or imposes a steric hindrance, which enables a discrimination between different prostaglandin receptor subtypes.

The IOP reducing effect of the new phenyl substituted PG analogues was investigated in cynomolgus monkeys using pneumatonometry. The pneumatonometer was calibrated for IOP measurement in monkeys using the closed stopcock method (50). Again the experimental eye was treated topically with the test compound while the contralateral eye received the vehicle only. It has to be emphasized that the IOP of the normotensive cynomolgus monkey is usually low, often around 10-14 mmHg, and consequently only pressure reductions of a few mmHg can be obtained. In spite of this, many of the analogues caused a clear-cut reduction in IOP (Fig. 3). Particularly analogues **5**, **8** and **11** were effective. These compounds were roughly equipotent with PGF_{2alpha}-ie. Least reduction was caused by the 15-epimers with the

Scheme 5

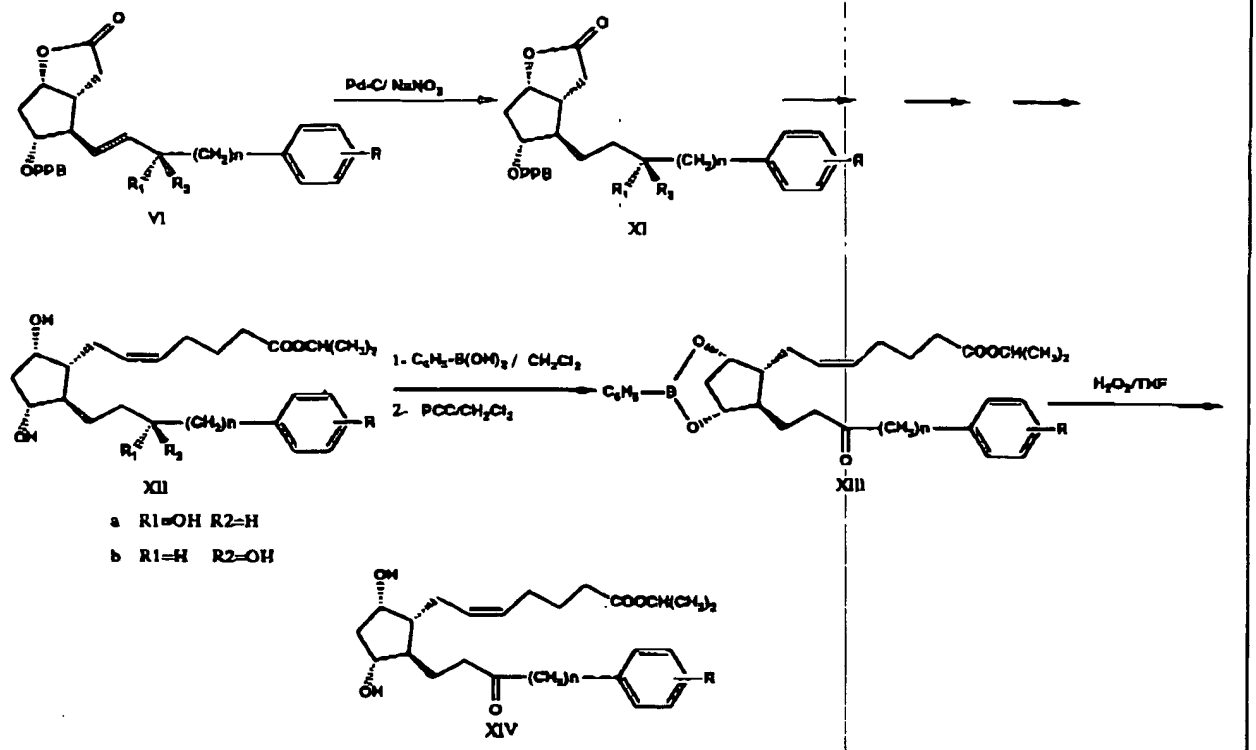


Table I: Maximum irritative and hyperemic responses of 17-phenyl trinor PGF_{2alpha}-ie analogues. PGF_{2alpha}-ie is included for comparison. All compounds were applied topically. The dose in cats was 1 mcg and in rabbits 0.5 mcg (n = 6; Mean +/- SEM).

Compound No.	Irritation in the cat eye (0 - 3)	Ocular surface hyperemia in rabbits (0 - 4)
PGF_{2alpha}-ie	2.7 +/- 0.2	2.9 +/- 0.2
5	0.0 +/- 0.0	1.5 +/- 0.3
6	0.0 +/- 0.0	1.1 +/- 0.3
7	0.0 +/- 0.0	1.4 +/- 0.2*
8	0.0 +/- 0.0	1.3 +/- 0.1*
9	0.0 +/- 0.0	0.7 +/- 0.1*
10	0.0 +/- 0.0	0.3 +/- 0.3*
11	0.0 +/- 0.0	0.6 +/- 0.3

*Dose 1.0 mcg; *n = 3.

15-OH group in the upward position **6**, **9** and by the 15-keto-17-phenyl-PGF_{2alpha}-ie analogues **7**, **10** (Fig. 3).

The log P values of the test compounds were computed using PACO program V 2:10 (Chemodata-Computer Chemie GmbH). Log P values of compounds **2** and **8** were determined experimentally using octanol/phosphate buffer (pH 7.4) and were found consistently to be about 1 log unit smaller than the computed values, indicating that the computed values give a satisfactory approximation of the true log P value. As can be seen from Table II the differences in log P values between compounds **5** to **11** were too small to account for significant differences in bioavailability, and thus the differences obtained in biologic activity in the eye must be considered to reflect the inherent properties of the analogues tested.

Repeated administration of compound **11** in laser treated ocular hypertensive monkeys caused a sustained reduction in IOP throughout the treatment period (51). Suprisingly, even if these analogues reduce IOP in monkeys they have very little effect on the IOP in cats (28) or rabbits (unpublished results).

The effects of the phenyl substituted prostaglandin analogues on the conjunctival blood vessels have been studied in the rabbit eye. Rabbits were treated topically on the eye with the prostaglandin analogues, and surface (mostly conjunctival and episcleral) hyperemia was documented by col-

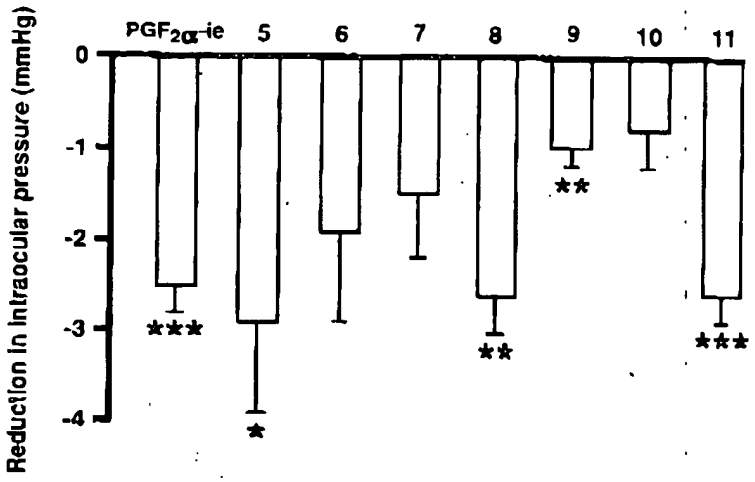
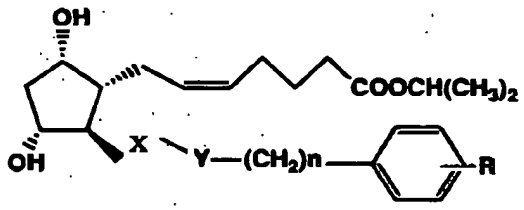


Fig. 3. Maximum intraocular pressure reducing effect of 1 mcg of 17-phenyl substituted PGF_{2alpha}-ie analogues in conscious cynomolgus monkeys after topical application. PGF_{2alpha}-ie included for comparison (n = 6; Mean +/- SEM). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$.

Table II: Calculated log P values of the test compounds.



Compound No.	X	Y	n	R	Log P*
5, 6	DB	C-OH	2	H	5.33
7	DB	C=O	2	H	5.00
8, 9, 11	SB	C-OH	2	H	5.57
10	SB	C=O	2	H	4.87
12	SB	C-OH	O	H	4.59
13	DB	C-OH	1	H	4.82
14	DB	C-OH	3	H	5.81
15	DB	C-OH	4	H	6.33
16	DB	C-OH	5	H	6.85
17	DB	C-OH	6	H	7.39
18	DB	C-OH	9	H	9.01
19	DB	C-OH	2	2-CH_3	5.64
20	DB	C-OH	2	4-CH_3	5.68
21	DB	C-OH	2	3-OCH_3	5.20
22	DB	C-OH	2	4-OCH_3	5.18
23	DB	C-OH	2	4-CF_3	6.24
24	DB	C-OH	2	4-F	5.39
2		PGF_{2alpha} isopropyl ester			5.47

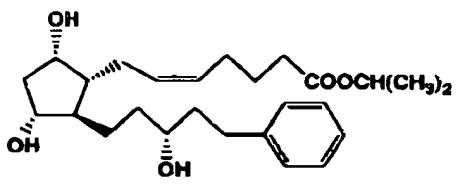
*Log P was calculated with PACO program V 2:10 (Chemodata-Computer Chemie GmbH) according to R.F. Rekker and C. Hansch. SB = Single Bond; DB = Double bond.

or photographs. The xperimental ye received the test compound while the contralateral eye served as a control receiving the vehicle only. The photographs were evaluat d on an arbitrary scale from 0-4. As can be seen in Table I, all phenyl substituted PGF_{2alpha}-ie analogues (Scheme 2) induced clearly less hyperemia than PGF_{2alpha}-ie. The analogues exhibiting least conjunctival hyperemia were generally those exhibiting least pharmacologic activity such as the earlier mentioned 15-OH epimers **6**, **9** and the 15-keto **7**, **10** 17-phenyl substituted prostaglandin analogues (Table I).

Latanoprost - A new drug candidate for glaucoma treatment

Latanoprost, (code name: PhXA41; 13,14-dihydro-17-phenyl-18,19,20-trinor-PGF_{2alpha} isopropyl ester; Scheme 6) **8** is the pure 15*R* epimer of compound **11** (PhXA34), which is a mixture of the 15*R* and 15*S* epimers in approximately equimolar proportion. Since the 15*S* epimer xerts similar biologic activity to the 15*R* epimer but is much weaker, exerting only about 10% of the activity of the 15*R* epimer (29) several of the pharmacological and clinical studies with PhXA41 have been carried out using the epimeric mixture (PhXA34). In this context it is pertinent to mention that the doses of PhXA34 used correspond roughly to 50% of equivalent doses of PhXA41. The reason for using PhXA34 in these first studies was that this substance originally was the drug candidate for a new prostaglandin based antiglaucoma drug.

Scheme 6



LATANOPROST

Preclinical studies

Pharmacodynamic studies performed in monkeys have demonstrated that the main mechanism of action of latanoprost to reduce IOP is by increasing the uveoscleral outflow, and no or very little effect has been seen on the conventional outflow of aqueous humor through Schlemm's canal (14). Of importance is that no negative effect was seen on the aqueous humor production (14). Thus, the reduction of IOP seen in primate eyes after topical administration of latanoprost is not due to a decrease in aqueous humor production as is the case with beta-adrenergic antagonists.

The effects of latanoprost on the ocular microcirculation have been carefully studied. Experiments performed with the radiolabelled microsphere technique (52) indicate that the acute effects of topically administered latanoprost in primate eyes are very modest, and the only tissues in which slight increase in blood flow could be detected were the anterior portion of the sclera and the ciliary body (14). In the same experiments the effect of latanoprost on capillary permeability was also determined using ^{125}I albumin, ^{131}I albumin and ^{51}Cr labelled erythrocytes according to well established techniques (53, 54). Topical administration of latanoprost in primate eyes had no effect on capillary permeability in any of the ocular tissues (14). Thus, these experiments show that latanoprost has negligible effects on the microcirculation in the eye. This is in contrast to PGF_{2alpha}-ie, which has been demonstrated to substantially increase the blood flow of the anterior segment upon topical application in monkeys (55).

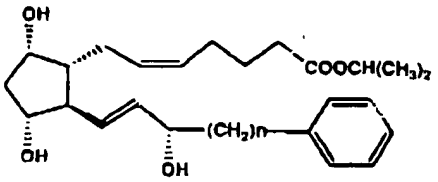
The prostaglandin receptor profile of latanoprost has been worked out *in vitro* using a receptor classification system previously described (56). In these experiments the free

*Table III: EC-50 values of latanoprost **8** in different prostaglandin receptor systems compared with the specific ligands.*

Receptor	Latanoprost EC-50 (moles/l)	Specific ligand: Substance	Specific ligand: EC-50 (moles/l)
FP	3.6×10^{-9}	PGF_{2alpha}	6.7×10^{-9}
EP_1	1.1×10^{-5}	PGE_2	3.3×10^{-8}
EP_2	3.6×10^{-4}	PGE_2	1.5×10^{-7}
EP_3	1.1×10^{-4}	Sulprostone PGE_1	1.8×10^{-10} 2.5×10^{-9}
DP and IP	3.4×10^{-3}	BW245C PGI_2*	4.7×10^{-8} 4.7×10^{-8}
TP	$>1.0 \times 10^{-3}$	U-46619**	3.1×10^{-6}

*Carbaprostacyclin used as stable ligand; **Stable T_xA_2 analogue.

Scheme 7



Name	COMPOUND No	n
15-PHENYL PGF*	12	0
16-PHENYL PGF	13	1
17-PHENYL PGF	5	2
18-PHENYL PGF	14	3
19-PHENYL PGF	15	4
20-PHENYL PGF	16	5
20-METHYLENE-PHENYL PGF	17	6
20-(4-PHENYL-BUTYL) PGF	18	9

*The compound was used as 13,14-dihydro-15-phenyl-pentanor $PGF_{2\alpha}$-ie

acid instead of the isopropyl ester has been used. Latanoprost has high affinity and selectivity for PGF_{2alpha} (FP) receptors as demonstrated in Table III (57). The affinity for EP2, EP3, DP, IP and TP receptors is very low compared with the specific ligands. However, the affinity for EP1 receptors is somewhat greater (57). These results indicate that FP receptors most likely are important in the mechanism leading to increased uveoscleral outflow and reduced IOP in primate and human eyes. Even if the site of action probably is the ciliary muscle and/or adjacent tissues, it is not fully clear which cells mediate the effect.

Clinical studies

In the clinical studies published so far mostly PhXA34 **11** has been used. It should be recalled that PhXA34 contains about 50% PhXA41. Several Phase II clinical trials are presently in progress with latanoprost. In a first Phase I study the ocular effects of PhXA34 were investigated in healthy human volunteers. PhXA34 was found to potently reduce IOP in a dose-dependent way with few side effects when applied topically on the eye (58). The concentrations tested were 0.003%, 0.01% and 0.03%. The only side effect observed was a conjunctival hyperemia which occurred with the highest concentration and which was much less pronounced than that generally seen with PGF_{2alpha}-ie. Repeated administration of the highest concentration, once daily for a total of 7 days in healthy volunteers caused a sustained reduction in IOP throughout the treatment period (58). There was no effect on the formation of aqueous humor as studied with fluorophotometry (58).

In a masked, placebo-controlled dose-finding study in patients with ocular hypertension a dose of approximately 3 mcg of PhXA34 per application corresponding to a concentration of 0.01% was found to be close to optimal with respect to maximum IOP reduction and a minimum of conjunctival hyperemia (59). This dose reduced IOP by an average of 30% from an initial pressure of around 25 mmHg without significant conjunctival hyperemia (59). In another masked placebo-controlled study ocular hypertensive patients were treated with 0.003% and 0.01% PhXA34 twice daily for 6 days (60). Both concentrations significantly reduced IOP with few side effects. The maximum reduction in IOP with the higher concentration was 10 mmHg from an initial pressure level of about 25 mmHg (60).

The longest duration of continuous treatment with latanoprost so far is 1 month (61). In this recent masked, placebo controlled multicenter study it was shown that ocular hypertensive patients treated with latanoprost exhibited reduced IOP throughout the treatment period (61). Thus, all clinical studies performed so far indicate that latanoprost (including PhXA34) effectively reduces IOP with markedly improved side effect profile compared to PGF_{2alpha} and its isopropyl ester. The duration of action of latanoprost in the eye is long, and a dose regimen of once a day application may be sufficient.

Variation of length of phenyl substituted om ga chain

As part of the structure-activity program in this project a series of phenyl substituted prostaglandin analogues with the omega chain length varying from 15-phenyl-pentanor-PGF_{2alpha}-ie to 20(4-phenyl-butyl)-PGF_{2alpha}-ie (Scheme 7) were synthetized and investigated for biologic activity in the eye. The synthetic pathway of these compounds is outlined in Scheme 4.

Structure-activity relationships

The purpose of these experiments was to study the influence of omega chain length on the potency and specificity of the phenyl substituted PGF_{2alpha}-ie analogues. The main emphasis has been on investigating general biologic activity and sensory irritative effect of the compounds. Thus, the cat eye has been used as a model, because exact measurements can be made on the miotic response reflecting general biologic activity and the cat eye is a satisfactory model for the sensory irritative effect. It should be stressed that most of the phenyl substituted PGF_{2alpha} analogues, as pointed out earlier, have a poor IOP reducing effect in cats. However, the miotic effect in cats expressing a FP receptor function seems to correlate with the IOP reducing effect in primates and man. All analogues have been used as isopropyl esters and they have been administered topically on the eye.

The miotic and irritative effects of a fixed dose (1 mcg) of phenyl substituted analogues of PGF_{2alpha}-ie with an omega chain ranging from 3 to 12 carbon atoms (total number of carbons 15-24) (Scheme 7) are illustrated in Figure 4. As can be seen, the position of the phenyl moiety in the omega chain is of fundamental importance for activity and selectivity. The 17-phenyl substituted analogu was ptimal because this compound exhibited high biologic activity without irritating effect. Again differences in partition coefficient between the compounds (Table II), and thus differences in bioavailability, cannot account for th striking differences in biologic activity. It can be s n that compounds **16**, **17** and **18** had higher log P values, but higher log P values reflect greater lipophilicity which usually enhances penetration into the eye. It should be observed that particularly the 16-phenyl **13** but also the 18-, 19-, 20-, and 20-methylene-ph enyl analogues **14**, **15**, **16**, **17** exhibited irritation although significantly less than PGF_{2alpha}-ie. Furthermore, it is noteworthy that the 16-phenyl analogue **13** of PGF_{2alpha}-ie at a dose of 1 mcg exerted no miotic effect at all. Decreasing the chain length to the 15-phenyl-pentanor analogue **12** resulted in loss of biologic activity at the dose level tested as could be expected and in general this compound is anticipated to have only weak activity. Elongation of the omega chain to the 2((4-phenyl-butyl)-PGF_{2alpha}-ie analogue **18** also resulted i.. loss of biologic activity in the eye, whereas the 20-methylene-phenyl analogue **17** exerted some biologic activity.

These results indicate that the 17-phenyl-18,19,20-trinor-PGF_{2alpha}-ie is unique in that this compound exhibits a structural conformation with no affinity for PG receptors it volved in the sensory irritative response (presumably PG receptors on sensory nerves), while retaining the affinity for FP receptors as demonstrated by the miotic response. In contrast, PGF_{2alpha}-ie analogues with shorter or longer phenyl substituted omega chain did show some affinity for PG receptors mediating nociceptive impulses. However, this affinity was much weaker than that of PGF_{2alpha}-ie. It appears that the steric hindrance of the phenyl ring and the interatomic distances between functional groups in the molecules are important for drug-receptor interaction.

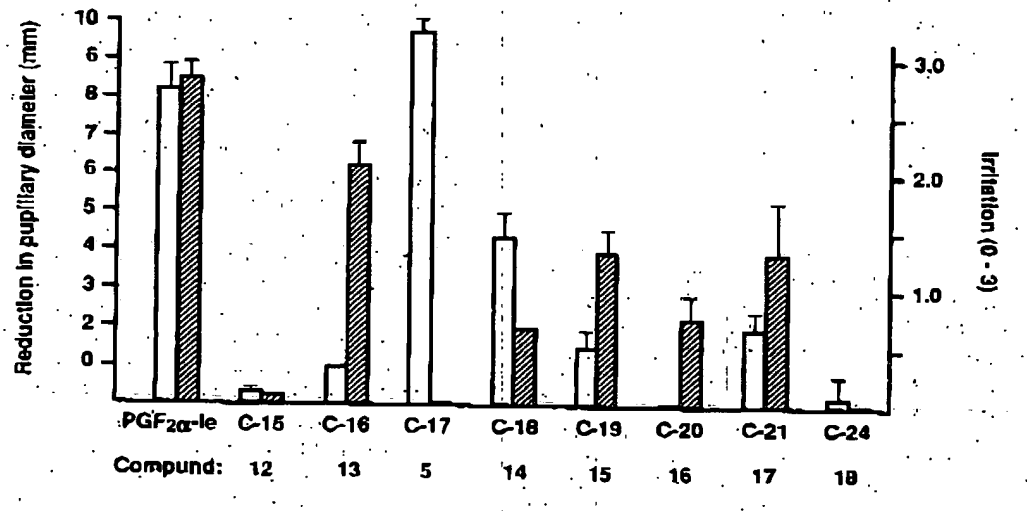


Fig. 4. Miotic and irritative effects of phenyl substituted PGF_{2alpha}-ie analogues, with different omega chain length. The compounds were administered topically on cat eyes and the dose was 1 mcg. The total number of carbon atoms in the analogues up to the phenyl ring is indicated below the abscissa. Empty columns indicate miosis and hatched columns ocular irritation. PGF_{2alpha}-ie included for comparison (n = 6; Mean +/- SEM).

Table IV: Miotic and irritative effects in the cat eye of 17-phenyl trinor prostaglandin analogues substituted in the phenyl ring. The compounds have been compared with PGF_{2alpha}-ie and compound 5. Reduction in pupil diameter was determined 3 h after treatment. Dose 1 mcg (n = 6; Mean +/- SEM).

Compound No.	Reduction in pupil diameter (mm)	Irritation (0 - 3)
PGF_{2alpha}-ie	-8.2 +/- 0.7	2.7 +/- 0.2
5	-9.7 +/- 0.3	0.0 +/- 0.0
19	-8.3 +/- 0.6	0.0 +/- 0.0
20	-2.5 +/- 0.2	0.0 +/- 0.0
21	-1.2 +/- 0.2	0.3 +/- 0.0
22	0.0 +/- 0.0	0.0 +/- 0.0
23	-0.5 +/- 0.2	0.0 +/- 0.0
24	-8.2 +/- 0.2	0.0 +/- 0.0

Effects of substituents on the phenyl ring

The biological effects of different substituents on the benzene ring of 17-phenyl-18,19,20-trinor-PGF_{2alpha}-ie have also been studied. The miotic and irritative effects in the cat eye were investigated as described above. The compounds investigated are shown in Scheme 8, and the synthesis of the compounds is outlined in Scheme 4.

Structure-activity relationships

The effect of introduction of a methyl group, methoxy group, trifluoromethyl group or fluorine into the phenyl ring on the structure-activity relationship has been studied. As evident from Table IV introduction of a methyl group into position 2 on the benzene ring **19** did not change the miotic or the irritative response. However, introduction of the methyl group into position 4 of the phenyl ring **20** markedly decreased the miotic effect (Table IV), probably reflecting a reduction of biologic activity based on steric hindrance.

Introduction of a methoxy group into the phenyl ring has been used to study the effect of an electron donating group in the vicinty of the benzene ring. Introduction of a methoxy group in position 3 of the phenyl ring **21** or in position 4 of the phenyl ring **22** resulted in markedly reduced miotic effect or complete loss of the miotic effect in cats (Table IV). Even ten times higher doses of compound **22** had very little miotic effect in the cat.

Introduction of a trifluoromethyl group into position 4 in the phenyl ring **23**, as can be expected, rendered the 17-phenyl-trinor-PGF_{2alpha}-ie analogue practically inactive (Table IV). Even in a dose of ten times that in Table IV very little miotic effect could be elicited. Introduction of electron donating or electron receiving groups such as methoxy and trifluoromethyl, respectively, conceivably gives this part of the molecule an ionic character that leads to decreased activity.

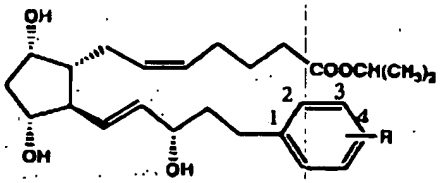
Introduction of fluorine into position 4 on the phenyl ring **24** did not appreciably change biologic activity as judged from the miotic response (Table IV). There was no irritating effect of this compound either.

Again, as evident from Table II, the differences in log P values between the compounds were not big enough to account for the significant differences in biologic activity between the compounds. These experiments thus show that it is possible to alter the biologic activity of 17-phenyl-18,19,10-trinor-PGF_{2alpha}-ie by introducing certain substituents on the phenyl ring.

Importance of ring structure on the omega chain

From what is mentioned above, it is evident that by substituting part of the omega chain of PGF_{2alpha}-ie with a phenyl ring, it is possible to totally eliminate the ocular irritating effect and to markedly reduce the hyperemic effect of

Scheme 8



COMPOUND No	POSITION OF SUBSTITUENT R	SUBSTITUENT R
19	2	CH3
20	4	CH3
21	3	OCH3
22	4	OCH3
23	4	CF3
24	4	F

PGF_{2alpha}-ie. Although a phenyl ring substitution seems to be particularly beneficial, substituti n with other ring structures such as cyclohexyl, thiophene and biphenyl also yields compounds with distinctly better side effect profile than that of PGF_{2alpha} and its prodrugs in the eye (unpublished results). Thus, PGF_{2alpha} possessing a terminal ring moiety on the omega chain exhibits a markedly improved therapeutic index in the eye.

Other phenyl substituted prostaglandin analogues

Several other prostaglandins have also been studied with respect to the effects of partial substitution of the omega chain with a phenyl ring. In general, similar changes in the biologic activity have been observed for instance with 17-phenyl substitution of PGE_2 and PGA_2, *i.e.*, a markedly improved side effect profile in the eye has been achieved (unpublished results). It is obvious that similar phenyl substitutions *e.g.*, of PGB_2, PGC_2 or PGD_2, can be anticipated analogously to improve the side effect profile of these prostaglandin analogues in the eye.

Conclusions

PGF_{2alpha} and its isopropyl ester have been shown to be potent ocular hypotensive agents in several animal species and in man. However, the frequent and disturbing side effects in the eye make it impossible to utilize PGF_{2alpha} as an ocular hypotensive agent clinically. Whereas the prodrug esters of PGF_{2alpha} do not significantly reduce the adverse effects in the eye, partial substitution of the omega chain with a phenyl ring dramatically reduces the ocular side effects of PGF_{2alpha}-ie. Such substitution totally eliminates the superficial irritating effect of PGF_{2alpha}-ie in the eye. This is probably due to a conformational change of the omega chain in the prostaglandin molecule, or steric hindrance, which enables a discrimination between different prostaglandin receptor subtypes. The most optimal chain length to which the ring structure is attached seems to be 5 carbon atoms (17-phenyl-18,19,20-trinor). The biologic activity of these compounds may further be altered by substitutions in the phenyl ring.

One of the most promising analogues **8** latanoprost is presently undergoing phase II clinical testing with encouraging results. This drug has been shown to potently reduce IOP in glaucoma patients with few side effects.

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